**Pharmacophore 1**

*Conformers setting*: Best setting (max. number conformers: 500)

*Data setting* for the 18 actives compounds:

* 5 as ignored (CID): for their kd<0.1

11213558,16725726,16722836,57397989,71717270

* 6 as test dataset (CID):

5329102,9926791,9813758,9977819,25126798,4455163

* 7 as training dataset

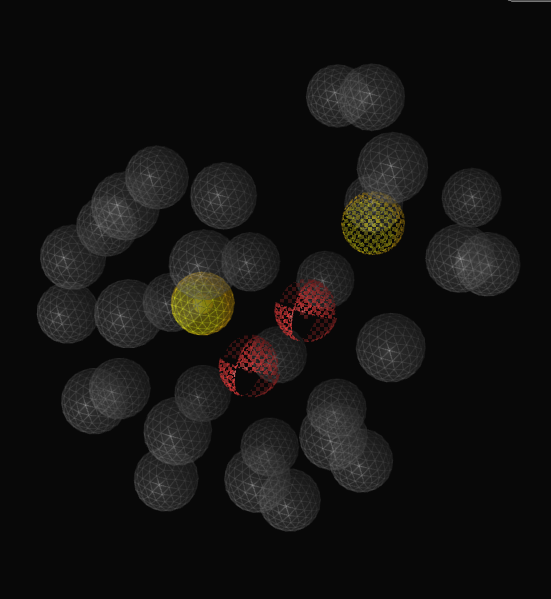
*Feature patterns*:

* 2 Hydrophobic centers (aromatic rings in center area with resonance)
* 2 Hydrogen Bond Acceptors (most of them are nitrogen or oxygen atoms in a ring)

*Pharmacophore 1 score*: 0.7241

In this pharmacophore, one compound (CID:25126798) in test dataset has the score of pharmacophore-fit as zero. It is the possible cause that makes this model no excelent score. In this compound, there is no center aromatic ring structure. This non-hydrophobic center leads to the consequence that even with many nitrogen in its ring structure, it doesn’t get pharmacophore-fit.

The ROC of “exclusion volume as disabled” has even more obvious negative value (Fig 3) than the one without that setting (Fig4). For the reason that “exclusion volume” in the model is structure base of pharmacophore, lacking the base of pharmacophore makes the ROC curve more not preferable but creates more hits (87 more hits out of 1034 compounds screening).

Fig 1: pharmacophore 1

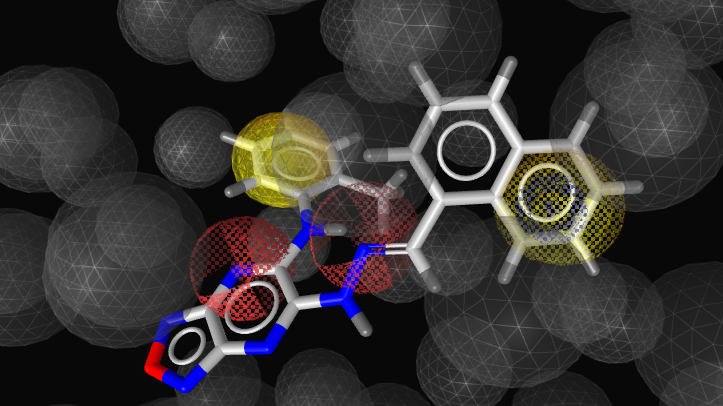


Fig 2: pharmacophore 1 with screening structure of PknB dataset

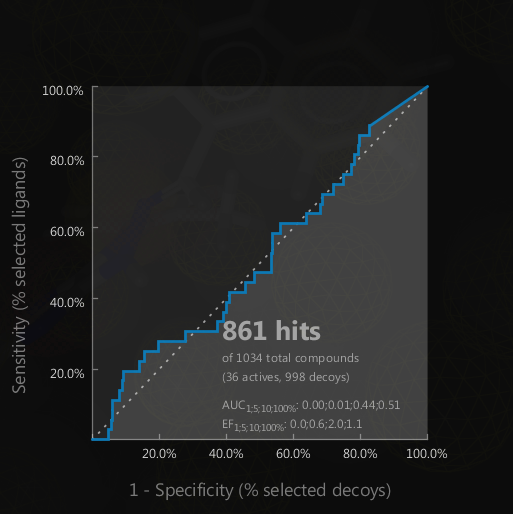


Fig 3: ROC curve of pharmacophore 1 with setting-exclusion volume-to be disabled

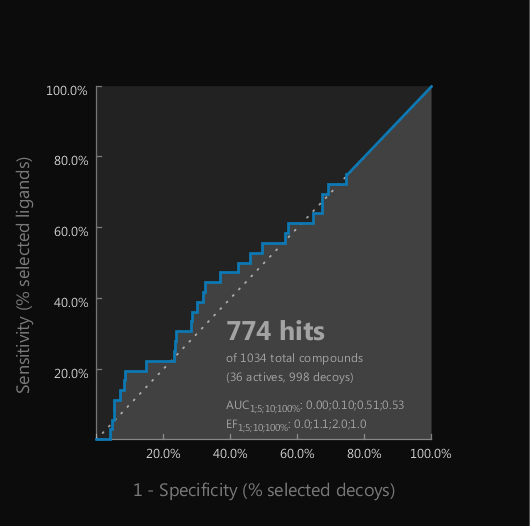


Fig 4: ROC curve of pharmacophore 1

**Pharmacophore 2**

*Conformers setting*: Best setting (max. number conformers: 500)

*Data setting* for 18 active compounds:

* 9 as test dataset (CID):

9926791,9813758,9977819,25126798,24905147,11427553,44299148,71717270,10138259

* 9 as training dataset (CID): for they have the max. number of conformers (500) 5329102,5328940,5494449,16038120,11213558,16725726,16722836,57397989,44551653
* 0 ignored

*Feature patterns*:

* 1 Hydrophobic centers (aromatic rings in center area with resonance)
* 2 Hydrogen Bond Acceptors (most of them are nitrogen or oxygen atoms in a ring)
* 1 Hydrogen Bond Donor (linker nitrogen-the nitrogen between two rings)

*Pharmacophore 2 score*: 0.6942

With different data setting, different phamacophore are generated. The speciality of this model is that it has the feature pattern – hydrogen bond donor (the biggest ball and its connected small ball with an arrow between them).

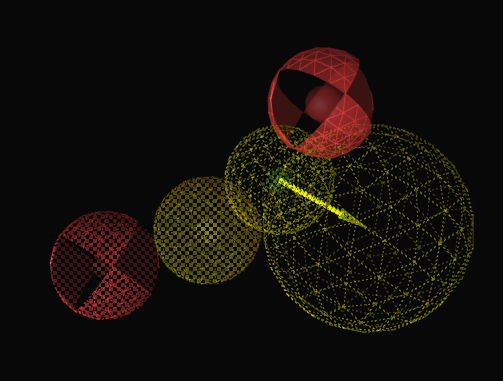


Fig 5: pharmacophore 2

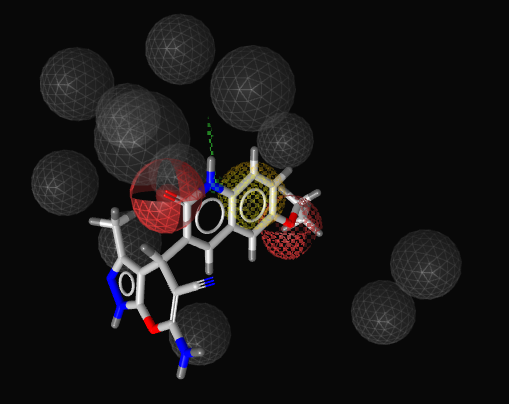


Fig 6: pharmacophore 2 with screening structure of PknB dataset

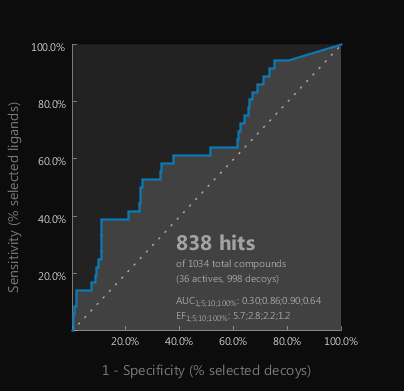


Fig 7: ROC curve of pharmacophore 2 with setting-exclusion volume-to be disabled

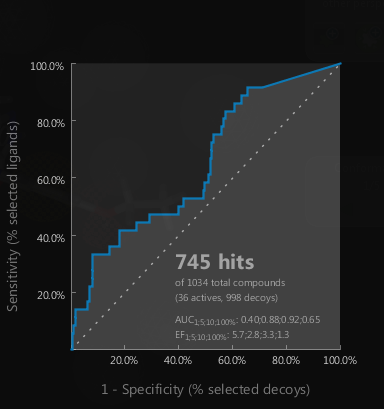


Fig 8: ROC curve of pharmacophore 2

**Pharmacophore 3**

*Conformers setting*: Best setting (max. number conformers: 500)

*Data setting* for 18 active compounds:

* 9 as test dataset (CID): choose randomly

9926791,9977819,25126798,24905147,71717270,5328940

* 9 as training dataset (CID): choose randomly 5329102,9813758,11213558,16722836,44551653,11427553,44299148,10138259
* 0 ignored (CID): choose randomly

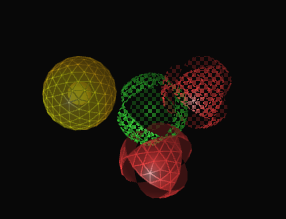
5494449,16038120,16725726,57397989

*Feature patterns*:

* 1 Hydrophobic centers (aromatic rings in center area with resonance)
* 2 Hydrogen Bond Acceptors (most of them are nitrogen or oxygen atoms in a ring)
* 1 Hydrogen Bond Donor (oxygen in hydroxyl group and nitrogen in linker/ring)

*Pharmacophore 3 score*: 0.7289

This pharmacophore has also a special feature pattern – hydrogen bond donor – like pharmacophore 2 does. But with different data setting, the contribution of the HB donor is also coming from different atoms. Here it’s the oxygen in the hydroxyl group in the side chain of the compounds that made it preserve HB donor feature. This contribution is not found in the previous model. The HB donor feature in this model is also distributed by nitrogen in the ring or in the linker position. Finally, pharmacophore score (0.72) is better than the previous one (0.69).

 Fig 9: pharmacophore 3 (hydrophobic-yellow; HB donor-green)

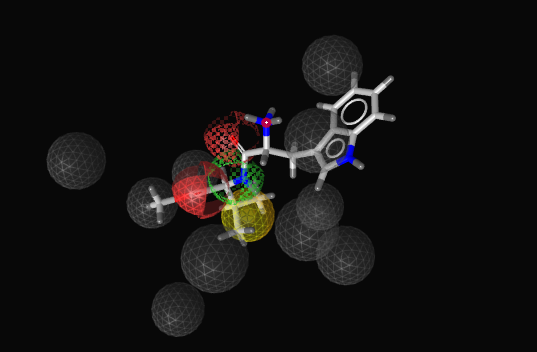


Fig 10: pharmacophore 3 with screening structure of PknB dataset

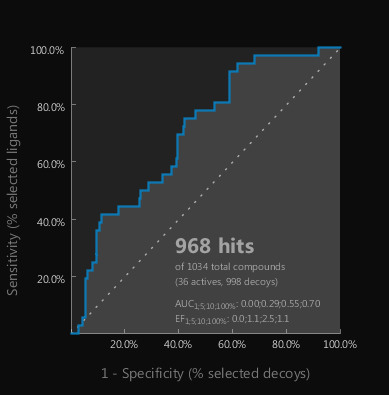
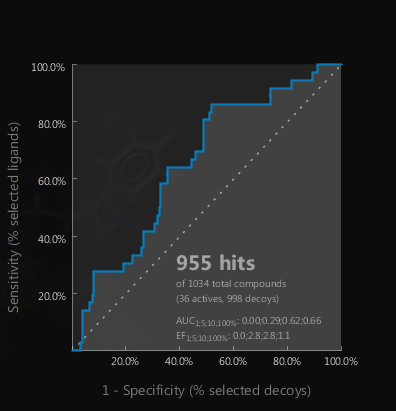


Fig 11: ROC curve of pharmacophore 3 with setting-exclusion volume-to be disabled

 Fig 12: ROC curve of pharmacophore 3

assignment 6-7 important feature in model:

The active compounds dataset we used to generate pharmacophore are [PknB assay from Mycobacterium tuberculosis](http://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=624753&loc=ea_ras) (AID: 624753). The test result of these 72 compounds, or said kinase inhibitors, shows that type 2 inhibitors are more selective than type 1. This result is reasonable for that type 2 inhibitor is non-competitive inhibitor which binds adjacently to the ATP site. The inhibition of type 2 inhibitor is executed by the conformational change of the kinase so that the enzyme can not perform its phosphorylation function. Since ATP is adenosine triphosphate and it is usually in negative charge in physiological pH environment, the active compounds should be positive charge so they can be close to each other and the inhibitor binds to the kinase. As a result, positive charge is an important feature in pharmacophore. However, it is also mentioned in the abstract of this bioassay that there are important exceptions to the observation that type 2 has better selectivity than type 1 does. This leads to the conclusion that there is something in common between type 1 and type 2 inhibitor so they can both show good inhibition activity. Then the hydrophobic feature in shows its importance in kinase inhibitor pharmacophore model. For the reason that kinase is part of biomolecules, it must contain a hydrophobic area to conduct its own/isolated biological reaction in its aquatic environment. In order to interact with the hydrophobic/active site of the kinase, the inhibitor should have hydrophobic center. In summary, hydrophobic center may be priority feature pharmacophore model building.

Reference:

1. Charlotte Harrison*. Nature Reviews Drug Discovery* 11, 21 (January 2012) | doi:10.1038/nrd3647.

2. L. Garuti, M. Roberti and G. Bottegoni, Non-ATP Competitive Protein Kinase Inhibitors